IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Antonius G. P. Oomens et al.	Confirmation No.: 9544
Application Serial No. 10/575,279	Group Art Unit: 1648
Filing Date: April 11, 2006	Examiner: Benjamin P. Blumel

For: RECOMBINANT VIRUSES WITH HETEROLOGOUS ENVELOPE PROTEINS

DECLARATION OF ANTONIUS G.P. OOMENS, Ph.D., UNDER 37 C.F.R. § 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Antonius G.P. OOMENS, Ph.D., hereby declare:

1. I am a co-inventor of the above-identified application.

2. I am presenting this Declaration to demonstrate that the claimed subject matter was invented in the U.S. before April, 2003.

3. Attached as Exhibit A is a section of laboratory notebook pages relating to an experiment entitled "Prepare simultaneously 381 and 392 stock to see if GP64 stabilizes RS." On this page, "stock" means a virus suspension; RS = human respiratory syncytial virus (HRSV); 381 = HRSV with glycoproteins deleted and substituted with GP64; 392 = wild-type HRSV with GFP marker gene inserted in place of a non-essential HRSV gene (i.e., control virus).

4. The fourth page of the Exhibit is dated January 3, 2003 ("1/3/03 Timepoint week 8"); the seventh page of the Exhibit is dated, for example, November 20, 2002 ("11/20/02").

5. This experiment was finished in early January, 2003. There is a graph clearly showing that 381, the recombinant virus with substituted GP64, remains stable over an 8-week time period whereas 392, the wild-type control, does not. The straight lines are the main experiment; the dotted lines are a duplication in which the samples were freeze-thawed prior to titrations. The results of the straight and dotted lines were the same, i.e., GP64–containing virus is much more stable. These

notebook pages demonstrate that the subject matter of the above-identified application had been conceived and reduced to practice before April, 2003.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the U.S. Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 1/15/09

signed: AGP Oomens

Antonius G.P. Oomens

[redacted]

Exhibit A

11/4 Prepare simultaneously 381 and 392 stock to examine if GP64 stabilizes Ki

Confluency O/N

~~• plated Vero @ 0.8 x 10^6 / 100 mm dish ~25%~~

Hz @ 1 x 10^6 / 100 mm dish ~35%

• 11/5: infect at 16.00:

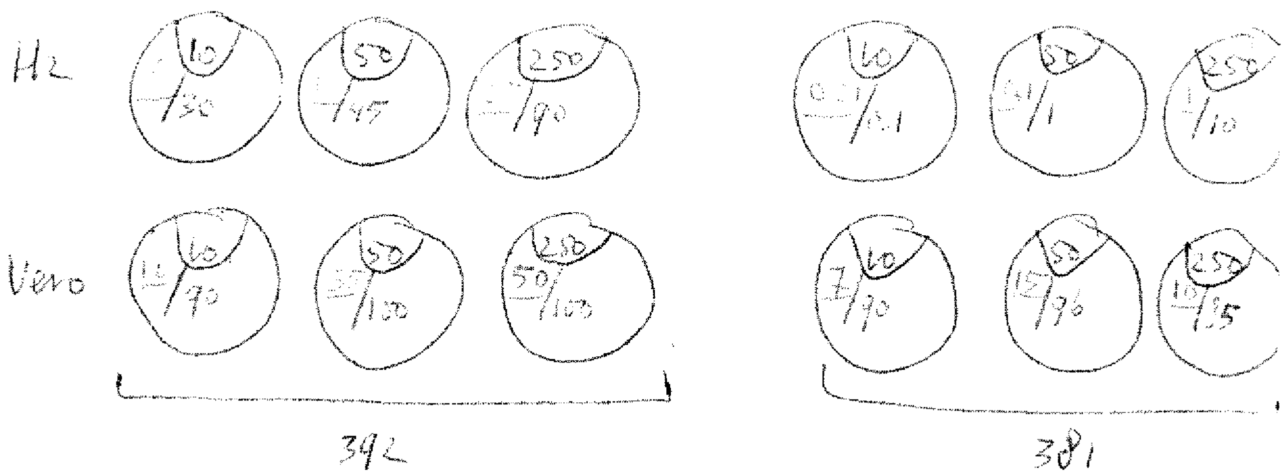
For 392 I used 8/20/01 G4 supt; titer is 7.1 x 10^6 (tubes 1,2)

For 381 I used 8/24/01 G4 supt; never titered or used before (tubes 6,

For each virus, I infected 3 100 mm dishes of Hz and Vero with 10 μl, 50 μl, or 250 μl virus

Virus was added directly to cell medium, and kept on.

Plates were put at 33°C.



• Score percentage infected cells on 11/8, 10.00: % in red

- Note the decrease of # open cells from 50 → 250 μl for 381

- Hz stock from 381 probably won't work

- For Vero: 381 10 or 50 μl and 392 10 μl are most comparable

→ remove 6 out of 10 ml and replace 5 ml fresh DMEM-5 or MEM 5. Volume now 8 ml. Keep at 33°C. (P.S. confluency of both cell types ~95% at this time)

• Score 11/10 in black

[illegible]

plate 10 and 50 μl (Hc) have groups of uninfected healthy Hc's. Seems like the best amount of virus may be between 50 and 250 μl.

Hc/381 : Most cells still in good shape, but getting overgrown. Percentage infectivity increasing but too low to get a good titer

⟹ Since neither 392 nor 381 is perfect, → discard the dishes and focus on Vero cells.

Vero / 392 : 10 μl appears lowest amount that still leads to near 100 % infection. Many syncytia present in the dishes, and medium has turned yellow.

Vero / 381 : 7 μl is best dish for same reason as Vero / 392 — and because 381 stock may contain many defective virus or very high particle : pfu ratio judged from the decrease of infectivity in the 250 μl plate. Medium in 381 dishes is also yellow.

⟹ Choose plates Vero – 10 μl for both to make stock for exp.

Processing

- Stocks were was on 14/10 at 12:00
- I reduced the supt from 7.5 → 6.2 ml and added 185 μl 1M hepes → bring to 30 mM hepes; color of med changed to pink. This was done at 13:15 (this is ~5 days)
- Dishes were further incubated from 13:15 to 17:30, then harvested 2 different ways to ensure that I will get a reasonable stock for both 381 and 392. For each virus:
  1. add another 185 μl 1M hepes (→ total to ~60 mM hepes)
  2. scrape cells into medium (~6.5 ml)
  3. Pipet 20x (and) then divide over 2 15 ml tubes
  4. put one tube at −80°C for 20 min and process the other as follows:
     - Pipet 20x (and) then spin 6 min 750 rpm at RT
     - Xfer supt to new tube. Discard cell pellet —

5. thaw the frozen tube and process exactly as the unfrozen one. Store tubes also at 4°C (~ 3 min in RT H_2O)

PS After aliquoting 100 μl amounts, I changed the order of tubes randomly. I labeled them 381, 381-f/t, 392 & 392-f/t

Tubes containing freeze-thawed virus are underlined: 381, 392

Titering

11/10 For time '0', I took samples from 4° between 22.00 and 24.00, (≈ 4-6 h after putting at 4°) one at a time, and titered them by TCID50

1. plated cells at 15.00 earlier in the day; density: 2500 U76 cells per well in 150 μl
2. set up 7 epp tubes and fill each with 180 μl (one tube will not get virus) DMEM3 + 50 mM hepes
3. add 900 μl DMEM3/hepes to the tube containing 100 μl virus; PUD extensively, then Xfer 20 μl to next tube (10^{-2})
4. vortex at setting 5 and repeat the procedure till 10^{-7}
5. Add virus dilutions to 96 well plates:
   - vortex again
   - electr. pipettor: speed 8, 15 × 10 μl
   - use 1st row as uninfected row

Samples titered: 2 × 381
2 × 381-f/t
3 × 392
3 × 392-f/t

11/11 For time '36', I took samples from 4° from 13.00-16.00 (?)
- plate [illegible] U76 per well in 150 μl for 96 well plate [illegible]
- Protocol same as that for timepoint '0' except
  • [illegible] μl [illegible] used to remove [illegible] from [illegible]
  • [illegible] 1st row [illegible] to [illegible] 100 μl [illegible] [illegible]

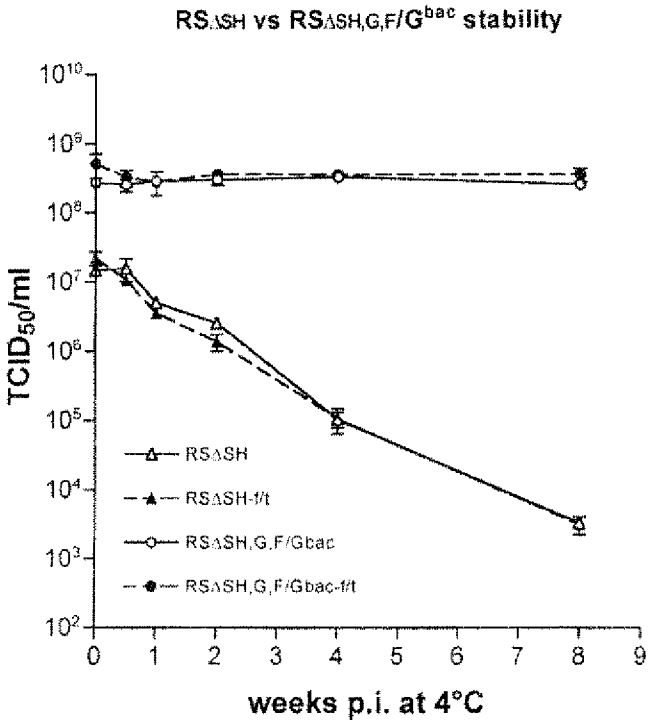
11/18 Timepoint 1 week: done early morning
- protocol exactly as that of time point 3rd.

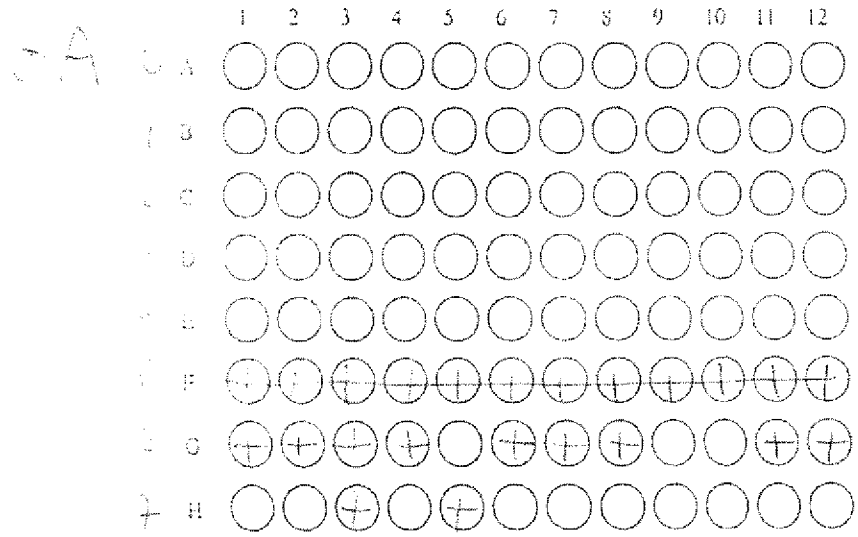
11/26 Timepoint 2nd week done early morning. This is actually
1 day late, however this should be okay

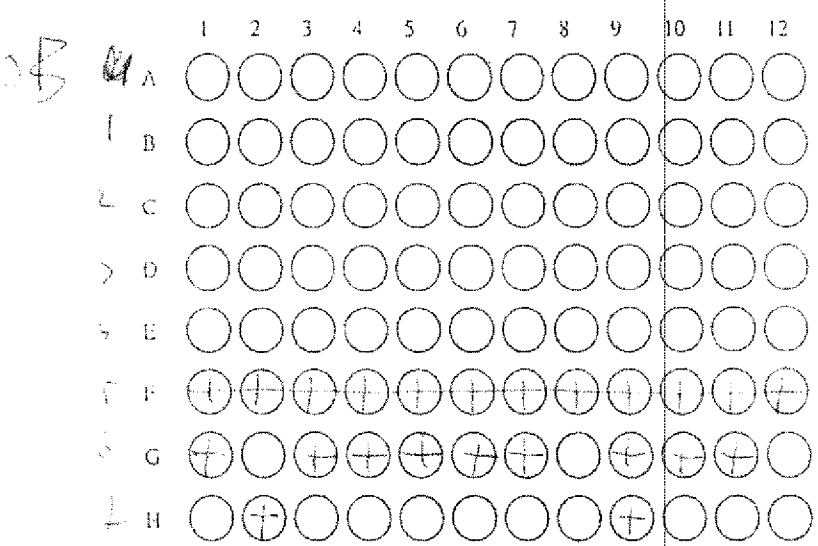
12/10 Timepoint week 4
All (3D, 3D-1/1, 3G, 3G-1/1) were done in triplicate this time

1/3/03 Timepoint week 8
same as 12/10

weeks		392	%	392-f/t	%	381	%	381-f/t	%
0	A	1.20 E7	81.47	3.16 E7	141.51	2.74 E8	100	3.16 E8	61.00
	B	1.61 E7	109.3	2.15 E7	96.28	2.74 E8	100	7.20 E8	139.00
	C	1.61 E7	109.3	1.39 E7	62.25	ND		ND	
average	abc	1.473 E7	100	2.233 E7	100	2.74 E8	100	5.18 E8	100.00
0.5	A	2.42 E7	164.29	1.00 E7	44.78	2.02 E8	73.72	2.42 E8	46.72
	B	1.87 E7	126.95	1.20 E7	53.74	3.16 E8	115.3	4.14 E8	79.92
	C	4.64 E6	31.5	1.00 E7	44.78	ND		ND	
1	A	4.64 E6	31.5	3.57 E6	15.99	3.98 E8	145.26	3.16 E8	61.00
	B	5.35 E6	36.32	3.98 E6	17.82	1.78 E8	64.96	2.51 E8	48.56
	C	5.27 E6	35.78	3.16 E6	14.15	ND		ND	
2	A	3.16 E6	21.45	7.20 E5	3.22	2.51 E8	91.61	3.57 E8	68.92
	B	2.74 E6	18.6	1.39 E6	6.22	3.51 E8	128.1	3.65 E8	70.46
	C	1.78 E6	12.08	2.02 E6	9.05	ND		ND	
4	A	1.39 E5	0.94	1.87 E5	0.84	3.98 E8	145.26	3.51 E8	67.76
	B	1.20 E5	0.81	5.62 E4	0.25	2.74 E8	100	3.16 E8	61.00
	C	5.27 E4	0.36	7.20 E4	0.32	3.16 E8	115.33	3.98 E8	76.84
8	A	3.98 E3	0.03	4.69 E3	0.02	2.02 E8	73.72	4.95 E8	95.56
	B	3.16 E3	0.02	1.61 E3	0.01	3.16 E8	115.33	3.16 E8	61.00
	C	2.80 E3	0.02	3.16 E3	0.01	2.42 E8	88.32	2.42 E8	46.72
12	A								
	B								
	C								
16	A								
	B								
	C								


RSΔSH vs RSΔSH,G,F/G^bac stability
TCID50/ml
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
0
1
2
3
4
5
6
7
8
9
weeks p.i. at 4°C
RSΔSH
RSΔSH-f/t
RSΔSH,G,F/Gbac
RSΔSH,G,F/Gbac-f/t





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e 11/10/22 – 1/10/26

3½ 11/14/22 – 1/20/26